PROTEASES AND OXIDANTS IN EXPERIMENTAL PULMONARY INFLAMMATORY INJURY

The investigators have exmined various biochemical parameters of pulmonary inflammation in experimental animals. Intrabronchial instillation of glucose oxidase-glucose (GO/G) to produce oxidants or formylated norleu-leu-phe (FNLP) or phorbol myristate acetate (PMA) as leukocytic stimuli induced severe acute pulmonary injury in New Zealand white rabbits. PMA also induced inflammation when administered intravenously. Each stimulus induced transudation of protein from the vascular space into the pulmonary tissues, and an influx of leukocytes during the 4-6 h period of the experiment. Pathophysiologic changes were measured by edema formation (transudation of ^{125}I-bovine serum albumin), and histologic examination. Biochemical analysis was performed by measuring concentrations of potentially injurious agents in bronchoalveolar lavage (BAL) fluid. Increased acid protease and myeloperoxidase levels were found in the BAL fluid after administration of either of the stimuli.

Schraufstätter, I. U., Revak, S. D., and *Cochrane, G. G.*

Journal of Clinical Investigation 73:1175-1184, 1984.

Other support: National Institutes of Health and the Office of Naval Research.

From the Department of Immunology, Scripps Clinic and Research Foundation, La Jolla, CA.

BIOCHEMICAL FACTORS IN PULMONARY INFLAMMATORY DISEASE

Various biochemical events taking place during pulmonary inflammation were examined in the bronchoalveolar lavage (BAL) fluids from patients with acute respiratory distress syndrome (ARDS) and in experimental animal models. In patients with ARDS, active neutrophil elastase was found in the BAL fluids. In these fluids, inactivation of the major elastase inhibitor α_1-protease inhibitor (α_1-PI) occurred. This was caused by oxidation of a methionine residue at the active site of the α_1-PI, and offered indirect evidence of oxidation occurring in the inflamed pulmonary tissues. Studies with experimental animals have been initiated to gain understanding of the relative roles of proteases, oxidants, arachidonate metabolites, complement and contact system components, and other mediators in the pathogenesis of pulmonary inflammation. Intrabronchial instillation of glucose oxidase/glucose to produce oxidants or formylated norleucylleucylphenylalanine or phorbol myristate acetate as leukocytic stimuli induced severe acute pulmonary injury in New Zealand white rabbits and rhesus monkeys. The injury was accompanied by leukocytic protease (acid cathepsins) release in rabbit lungs and oxidant formation, and could be inhibited by neutrophil depletion. Oxidant formation was demonstrated by the inactivation of catalase by 3-amino-1,2,4-triazole in the presence of H_2O_2, a drop in intracellular glutathione levels, and in the rhesus monkey by inactivation of α_1-PI.

Schraufstätter, I., Revak, S. D., and *Cochrane, C. G.*

Federation Proceedings 43:2807-2810, 1984.

Other support: National Institutes of Health and the Office of Naval Research.

From the Department of Immunology, Research Institute of Scripps Clinic, La Jolla, CA.

CERULOPLASMIN: INCRI
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Galdston, M. et al.

American Review of Respir

Other support: The Louis

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CERULOPLASMIN: INCREASED SERUM CONCENTRATION AND IMPAIRED ANTIOXIDANT ACTIVITY IN CIGARETTE SMOKERS, AND ABILITY TO PREVENT SUPPRESSION OF ELASTASE INHIBITORY CAPACITY OF ALPHA$_1$-PROTEINASE INHIBITOR

Bronchoalveolar lavage fluid of smokers and nonsmokers contains significant concentrations of ceruloplasmin, the major serum inhibitor of lipid peroxidation, with limited superoxide dismutase activity. This suggested that ceruloplasmin may protect the lower respiratory tract against oxidant(s) in cigarette smoke and air pollutants. To follow up on this, these investigators studied (1) serum ceruloplasmin concentration and antioxidant activity (percentage inhibition of autoxidation of ox-brain homogenate) in healthy male and female smokers and nonsmokers, and (2) the capacity of ceruloplasmin to prevent suppression of the elastase inhibitory capacity of alpha$_1$-proteinase inhibitor by the oxidant chloramine T and by cigarette smoke solution. Mean ceruloplasmin concentration was significantly higher in smokers than in nonsmokers and in females than in males whether or not they smoked. Serum antioxidant activity showed significant linear correlations with serum cerulaplasmin in smokers and nonsmokers of both sexes. There was a linear relationship between ceruloplasmin concentration and its ability to prevent suppression of the elastase inhibitory capacity of alpha$_1$-proteinase inhibitor by chloramine T and cigarette smoke solution. These findings indicate (1) that cigarette smoking can cause partial inactivation of serum antioxidant activity accompanied by insufficient compensatory increase in ceruloplasmin concentration, and (2) that ceruloplasmin may protect the lung against oxidant(s) in cigarette smoke and air pollutants.

Galdston, M. et al.

American Review of Respiratory Disease 129:258-263, 1984.

Other support: The Louis and Rose Klosk Fund.

From the Department of Medicine and the Chest Service, Bellevue Hospital, and the Department of Environmental Medicine, New York University Medical Center, New York.

CIGARETTE SMOKE-INDUCED BRONCHOCONSTRICTION IN DOGS: VAGAL AND EXTRAVAGAL MECHANISMS

The authors of this paper studied the mechanism of cigarette smoke-induced bronchoconstriction by methods that allowed separation of vagal afferent and efferent routes. To evaluate the severity of smoke-induced bronchoconstriction in anesthetized dogs, they measured airway pressure and airflow resistance (Rrs, forced oscillation method). They studied the mechanisms in other dogs by measuring airway pressure, central airway smooth muscle tone in tracheal segments *in situ*, and respiratory center drive by monitoring phrenic motor nerve output, including the role of vagal and extravagal nerves vs. the role of blood-borne materials during inhalation of cigarette smoke. Rrs increased more than fourfold with smoke from one cigarette delivered in two tidal volumes. About half the airway response was due to local effects of smoke in the lungs. The remainder was due to stimulation of the respiratory center, which activated vagal motor efferents to the airway smooth muscle. Of this central stimulation, about half was due to blood-borne materials and the rest to vagal pulmonary afferents from the lungs. These investigators conclude that inhalation of cigarette

smoke in dogs causes severe bronchoconstriction which is mediated mainly by extravagal mechanisms.

Hartiala, J., Mapp, C., Mitchell, R. A., Shields, R. L., and *Gold, W. M.*

Journal of Applied Physiology: Respirat. Environ. Exercise Physiol. 57(4):1261-1270, 1984.

Other support: National Heart, Lung and Blood Institute.

From the Cardiovascular Research Institute, Departments of Medicine and Physiology, University of California, San Fransisco.

LOCALIZATION OF CALMODULIN IN DIFFERENTIATING PULMONARY TYPE II EPITHELIAL CELLS

Pulmonary surfactant, a complex mixture of lipid, protein, and carbohydrate which lines alveolar surfaces, is synthesized by alveolar type II pneumocytes and stored in inclusions called lamellar bodies. In the present study the researchers have investigated the role of the calcium-binding protein, calmodulin, in regulating surfactant secretion in differentiating rat fetal type II pneumocytes. Lamellar body secretion is stimulated in differentiating type II cells *in vitro* by the calcium ionophore, A23187. A23187-induced secretion is blocked by the phenothiazine drugs, trifluoperazine and chlorpromazine, but is unaffected by the inactive analogs, trifluoperazine sulfoxide and chlorpromazine sulfoxide. Immunofluorescence studies on cultured type II pneumocytes show that the percentage of Nomarski-dense intracellular granules, which stain positively with anticalmodulin antibody, increases when the cells are stimulated with the calcium ionophore, A23187. Since these Nomarski-dense granules are positively stained by phosphine-3R, these results indicate that increased amounts of immunoreactive calmodulin appear associated with lamellar body surfaces when the cells are stimulated for secretion. In addition, ultrastructual localization of calmodulin on isolated lamellar bodies using protein A-colloidal gold indicates that calmodulin is present on their outer surfaces. Taken together, these and other results implicate calmodulin in pulmonary surfactant secretion.

Hill, D. J., Wright, T. C., Jr., Andrews, M. L., and *Karnovsky, M. J.*

Laboratory Investigation 51(3):297-306, 1984.

Other support: National Cancer Institute.

From the Department of Pathology, Harvard Medical School, Boston.

SEROTONIN AND THE PULMONARY CIRCULATION

Although serotonin (5-hydroxytryptamine, 5-HT) has been discredited as the mediator of hypoxic pulmonary vasoconstriction, it continues to be considered one of the vasoconstricting substances and has been demonstrated in the lungs of all species investigated. In summarizing earlier studies, evidence has been offered that removal of 5-HT is inhibited by hypothermia or hyperoxia, is not influenced by hypoxia, is inhibited by anoxia (which should be considered separately from physiologic hypoxia), and is also inhibited by cyanide, imipramine, chlorpromazine, and ouabain, among others. These kinetic and inhibitor studies support the criteria for active trans-

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port. The work reported in this chapter originates from earlier studies of pharmacologic modification of pulmonary arterial function and morphology. As part of an investigation of the effects of selected systemic vasodilators on the pulmonary circulation, ketanserin (R41468) and *dl*-p-chlorophenylalanine (*p*-CPA) were tested. A third study was initiated to confirm or reject the results of the earlier ketanserin study and contrast its activity with another 5-HT_2 inhibitor, cyproheptadine (Periactin). In summary, these studies support the previously reported suggestion that the pathogenetic mechanism of RV hypertrophy and medial thickening in chronic hypoxia may not be the same. Furthermore, serotonin seems to exhibit both excitatory and inhibitory activities on pulmonary arteries of 50 to 100 μM in size. This effect was seen by either depleting 5-HT with *p*-CPA treatment or by blocking the 5-HT_2 receptors with ketanserin. These experiments focuses attention on new concepts and possibilities for the control of the pulmonary circulation and is compatible with the hypothesis that normoxic pulmonary vasodilation is the active phase of the pulmonary vascular response to hypoxia.

Will, J. A., *Keith, I. M.*, Buckner, C. K., Chacko, J., Olson, E. B., Jr., and Weir, E. K.

In: Becker, K. L. and Gazdar, A. F. (eds.): *The Endocrine Lung in Health & Disease*, Philadelphia: W. B. Saunders, 1984, pp. 137-154.

Other support: College of Agricultural and Life Sciences, Air Force, and the National Institutes of Health.

From the Department of Veterinary Science, University of Wisconsin, Madison.

VASCULAR PROTEIN LEAKAGE IN VARIOUS TISSUES INDUCED BY SUBSTANCE P, CAPSAICIN, BRADYKININ, SEROTONIN, HISTAMINE AND BY ANTIGEN CHALLENGE

In the work reported here: (1) Plasma extravasation was induced in rats or guinea pigs by intravenous injections of 1. substance P (SP), 2. the C-terminal SP-hexapeptide SP(6-11), 3. serotonin (5-HT), 4. histamine, 5. bradykinin, 6. capsaicin and 7. by antigen challenge. (2) Plasma extravasation induced by SP, SP(6-11), by 5-HT and by capsaicin was, with few exceptions, observed in the same tissues. The effect of SP was not blocked by H_1 and H_2 histamine receptor antagonists. The effect of i.v. capsaicin was absent in capsaicin desensitized animals. Plasma extravasation upon i.v. SP, SP(6-11), 5-HT and capsaicin was seen in the skin and in all organs containing mucus membranes except the intestinal mucosa, and (3) Plasma extravasation by histamine, bradykinin, and antigen challenge of sensitized guinea pigs was also observed in the stomach and intestine. Plasma extravasation and bronchoconstriction by antigen challenge with 20 μg/kg ovalbumin was completely blocked by combined H_1 and H_2 histamine receptor blockade. Both responses were reduced to about the half capsaicin desensitized guinea pigs, though the reduction of the permeability response was statistically not significant in all organs. In conclusion, several substances including anaphylaxis induce protein leakage in many tissues with differing selective distribution patterns. Anaphylactic histamine release leads to protein leakage partly via activation of sensory neurons. SP is a likely mediator of neurogenic protein leakage in many organs.

Saria, A., *Lundberg, J. M.*, Skofitsch, G., and Lembeck, F.

Naunyn-Schmiedeberg's Archives of Pharmacology 324:212-218, 1983.

Other support: Austrian Scientific Research Fund, Swedish Medical Research Council, Swedish Tobacco Company, Wilbergs Stiftelse, Hans och Loo Ostermans Stiftelse, Gustav V Foundation, Svenska Läkaresällskapet, Magnus Bergvalls Stiftelse, Augusta and Petrus Hedlungs Stiftelse, and Astra Foundation.

From the Department of Experimental and Clinical Pharmacology, University of Graz, Graz, Austria, and the Department of Pharmacology, Karolinska Institutet, Stockholm, Sweden.

SUBSTANCE P AND CAPSAICIN-INDUCED CONTRACTION OF HUMAN BRONCHI

In the present study the effects of substance P (SP) and capsaicin on human bronchial smooth muscle tone were monitored *in vitro*. Results showed that SP induced a dose-dependent contraction of human segmental bronchi *in vitro* with a threshold dose of about 10^{-9} M. These preparations were obtained from patients undergoing lung tumor surgery. The SP-induced contractions were resistant to mepyramine and atropine, suggesting a direct effect on the bronchial smooth muscle. Capsaicin (10^{-5} M) also induced a slowly developing, strong atropine-resistant contraction of human bronchi *in vitro*. A rapid tachyphylaxis developed for the response to capsaicin. Both SP and capsaicin were less potent than acetylcholine and histamine in inducing contractions of human bronchi. This finding may, however, be partly due to the experimental conditions; both SP and capsaicin were comparatively much more potent in guinea-pig preparations. Transmural field stimulation of the bronchial preparations in man resulted in contractions that were largely sensitive to atropine. The presence of capsaicin-induced bronchial contractions, however, indicates the existence of a local noncholinergic axon-reflex control of bronchial smooth muscle tone by SP in man.

Lundberg, J. M., Martling, C-R. and Saria, A.

Acta Physiologica Scandinavica 119:49-53, 1983.

Other support: Swedish Medical Research Council, Swedish Tobacco Company, Wibergs Stiftelse, Hans och Loo Ostermans Stiftelse, Gustaf V Foundation, Karolinska Institutet Fonder, Svenska Läkaresällskapet, Magnus Bergvalls Stiftelse, Austrian Scientific Research Fund, Petrus and Augusta Hedlunds Fund, and Astra Foundation.

From the Department of Pharmacology, Karolinska Institutet, and Department of Anesthesiology, Karolinska Hospital, Stockholm, Sweden; and Department of Experimental and Clinical Pharmacology, University of Graz, Graz, Austria.

EFFECTS AND DISTRIBUTION OF VAGAL CAPSAICIN-SENSITIVE SUBSTANCE P NEURONS WITH SPECIAL REFERENCE TO THE TRACHEA AND LUNGS

The origin of substance P (SP)-immunoreactive neurons in the lower respiratory tract, esophagus and heart of guinea pigs was demonstrated in some of these studies by surgical denervation or capsaicin pretreatment with subsequent determination of the tissued levels of SP by radioimmunoassay. In other experiments the effect of vagal nerve stimulation on the SP levels in these tissues was studied. The effects of cap-

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saicin-sensitive afferents in the respiratory tract mucosa and bronchial smooth muscle was also studied by analysis of vascular permeability to Evans blue and insufflation-pressure changes. The present data indicate that all SP nerves in the trachea and lung are afferent and capsaicin-sensitive. The trachea and stem bronchi receive SP afferents mainly from the right vagus nerve with cell bodies located in both the nodose and jugular ganglia. The SP innervation of the lung seems to have a dual origin: 1. Afferents from both vagal nerves with a crossed type of innervation pattern; 2. A non-vagal source which consists of about 40% of the SP nerves in the lung. These nerves probably originate from thoracic spinal ganglia. The effects of ether and capsaicin on insufflation pressure and increase in vascular permeability were dependent on the integrity of capsaicin-sensitive afferents of both vagal and non-vagal origin. In the guinea pig, systemic capsaicin pretreatment to adult animals seemed to result in irreversible changes in the respiratory tract, while in the rat a successive recovery of the functional response of capsaicin-sensitive afferents occurred.

Lundberg, J. M., Brodin, E. and Saria, A.

Acta Physiologica Scandinavica 119:243-252, 1983.

Other support: Swedish Medical Research Council, Swedish Tobacco Company, Wibergs Stiftelse, Hans och Loo Ostermans Stiftelse, Gustaf V Foundation, Astra Foundation, Magnus Bergvalls Stiftelse, Karolinska Institutet Forskningsfonder and Svenska Läkaresällskapet.

From the Department of Pharmacology, Karolinska Institutet, Stockholm, Sweden.

EFFECT OF ROUTE OF ATROPINE DELIVERY ON BRONCHOSPASM FROM COLD AIR AND METHACHOLINE

The authors undertook a study to determine whether the apparent disparity between the dose of inhaled atropine required to inhibit bronchoconstriction induced by inhaled methacholine and the dose required to inhibit the bronchoconstriction induced by eucapnic hyperpnea with cold air is a function of the route of administration of atropine. In six subjects with asthma, they constructed dose-response curves to inhaled methacholine and to eucapnic hyperpnea with cold air after treatment with inhaled atropine (0.5 mg delivered) and intravenous placebo, with inhaled placebo and intravenous atropine (0.5 mg injected), and with inhaled and intravenous placebos. Atropine by either route shifted the dose-response curves to both cold air and to methacholine to the right. In every subject, however, inhaled atropine caused a markedly greater rightward shift of the inhaled methacholine dose-response curve than did intravenous atropine, whereas inhaled and intravenous atropine had similar effects on the cold air dose-response curve. These findings suggest that the apparent disparity between the doses of atropine required to inhibit methacholine- and cold air-induced bronchoconstriction may be a function of the route of administration of atropine and thus does not imply a nonmuscarinic action of atropine. These findings support the view that cold air causes bronchoconstriction via muscarinic pathways.

Sheppard, D., Epstein, J., Holtzmen, M. J., *Nadel, J. A.*, and Boushey, H. A.

Journal of Applied Physiology: Respirat. Environ. Exercise Physiol. 54(1):130-133, 1983.

Other support: National Heart, Lung, and Blood Institute.

From the Cardiovascular Research Institute and Departments of Medicine and Physiology, University of California, San Francisco, and the Medical Service, San Francisco General Hospital, San Francisco.

CHARACTERIZATION OF *BETA* ADRENOCEPTOR SUBTYPES IN CANINE AIRWAY SMOOTH MUSCLE BY RADIOLIGAND BINDING AND PHYSIOLOGICAL RESPONSES

These researchers have investigated tracheal smooth muscle of the dog and have used [^{3}H] DHA to study the characteristics of *beta* receptors in homogenates of this tissue. For comparison, they also studied *in vitro beta* adrenergic responses in the same tissue using both exogenous *beta* agonists and electrical stimulation of sympathetic nerves. Specifically, *beta* adrenoceptor subtypes in canine tracheal smooth muscle have been investigated by radioligand binding and by physiological responses to *beta* agonists and sympathetic nerve stimulation *in vitro*. Specific binding of [^{3}H] dihydroalprenolol to tracheal smooth muscle membranes was of high affinity (K_d = 1.0 ± 0.08 nM), as in peripheral lung membranes from the same animals, but the concentration of binding sites (95.6 ± 4.7 fmol/mg of protein) was much lower in lung (532 ± 48 fmol of protein). Binding was stereoselective and agonists competed with the rank order of potency isoproterenol>epinephrine>norepinephrine, signifying a preponderance of *beta*-2 receptors. Using selective *beta* antagonists, the researchers determined the ratio of *beta*-1/*beta*-2 receptors in tracheal smooth muscle membranes to be 1:4. These and other related results suggest that most *beta* receptors in dog tracheal smooth muscle are of the *beta*-2 subtype and mediate responses to circulating catecholamines, but there is a small concentration of *beta*-1 receptors that mediate the response to neurally released norepinephrine.

Barnes, P. J., *Nadel, J. A.*, Skoogh, B-E., and Roberts, J. M.

The Journal of Pharmacology and Experimental Therapeutics 225(2):456-461, 1983.

Other support: National Institutes of Health.

From the Cardiovascular Research Institute and Departments of Medicine and Physiology, University of California, San Francisco.

SELECTIVE GENERATION OF LEUKOTRIENE B_4 BY TRACHEAL EPITHELIAL CELLS FROM DOGS

Infiltration by neutrophils is a predominant histologic feature of acute inflammatory responses in pulmonary airways. The recent demonstration that neutrophil infiltration was localized predominantly to the epithelial layer of the airway wall in dogs breathing ozone suggested that critical inflammatory mediators were released from the epithelial cells. In the work reported here, the incubation of suspensions of canine tracheal epithelial cells of greater than 95% purity with arachidonic acid (25-200 μg/ml) for 60-120 min resulted in the generation of a maximum of 36.2 ± 9.1 picomoles of leukotriene B_4/10^6 cells, less than 2.0 picomoles of leukotrienes C_4, D_4, and E_4/10^6 cells, and 1030 ± 463, 767 ± 500, and 324 ± 100 picomoles/10^6 cells of 15-, 12-, and 5-hydroxy-eicosatetraenoic acids, respectively (mean ± SEM, n = 8). The identity of leukotriene B_4 was established by chromatographic and spectral properties, by reactivi-

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ty with mono-specific anti-plasma and by the chemotactic activity for neutrophils. Thus, the epithelium may be an important source of mediators of inflammation and hypersensitivity of pulmonary airways.

Holtzman, M. J., Aizawa, H., *Nadel, J. A.*, and Goetzl, E. J.

Biochemical and Biophysical Research Communications 114(3):1071-1076, 1983.

Other support: National Institutes of Health and the California Air Resources Board.

From the Cardiovascular Research Institute, Howard Hughes Medical Institute, and the Departments of Medicine and Physiology, University of California, San Francisco.

IMPORTANCE OF AIRWAY INFLAMMATION FOR HYPERRESPONSIVENESS INDUCED BY OZONE

As summarized in this paper, the authors studied whether ozone-induced airway hyperresponsiveness correlates with the development of airway inflammation in dogs. To assess airway responsiveness, the researchers determined increases in pulmonary resistance produced by delivering acetylcholine aerosol to the airways. To assess airway inflammation, they biopsied the airway mucosa and counted the number of neutrophils present in the epithelium. Airway responsiveness and inflammation were assessed in anesthesized dogs before ozone exposure and 1 h and 1 wk after ozone (2.1 ppm, 2 h). Airway responsiveness increased markedly at 1 h after ozone and returned to control levels 1 wk later in each of 6 dogs, but responsiveness did not change after ozone in another 4 dogs. Furthermore, dogs that became hyperresponsive also developed a marked and reversible increase in the number of neutrophils in the epithelium, whereas dogs that did not become hyperresponsive had no change in the number of neutrophils. For the group of dogs, the level of airway responsiveness before and after ozone exposure correlated closely with the number of epithelial neutrophils. The results suggest that ozone-induced airway hyperresponsiveness may depend on the development of an acute inflammatory response in the airways.

Holtzman, M. J., Fabbri, L. M., O'Byrne, P. M., Gold, B. D., Aizawa, H., Walters, E. H., Alpert, S. E., and *Nadel, J. A.*

American Review of Respiratory Disease 127:686-690, 1983.

Other support: National Heart, Lung and Blood Institute, Fisons Corporation and the California Air Resources Board.

From the Cardiovascular Research Institute and Department of Medicine, University of California, San Francisco.

AUTORADIOGRAPHIC LOCALIZATION OF AUTONOMIC RECEPTORS IN AIRWAY SMOOTH MUSCLE

Using experimental conditions that proved to be optimal for specific receptor binding, these investigators have studied the distribution of alpha-adrenergic, beta-adrenergic and muscarinic receptors in smooth muscle of airways from trachea to terminal bronchioles. Autoradiographic methods were used to determine the distribu-

tion of autonomic receptors in airway smooth muscle of ferret from trachea to terminal bronchioles; [^{3}H] dihydroalprenolol, [^{3}H] prazosin, and [^{3}H] quinuclidinyl benzilate were used to label beta-adrenergic, alpha-adrenergic, and muscarinic receptors, respectively, using experimental conditions that gave maximal specific receptor binding. Marked differences were found in the longitudinal distribution of each receptor and in distribution of the various receptors in each caliber airway. Beta-receptors were present in high density throughout the airways, with the highest density in bronchioles. Alpha-receptors were sparse in large airways but numerous in small bronchioles, whereas cholinergic receptors were numerous in bronchial smooth muscle, sparse in proximal bronchioles and almost absent from distal bronchioles. This method may be useful in studying alterations of autonomic receptor distribution in small and large airways after experimental manipulation and in disease.

Barnes, P. J., Basbaum, C. B. and *Nadel, J. A.*

American Review of Respiratory Disease 127:758-762, 1983.

Other support: National Institutes of Health.

From the Cardiovascular Research Institute and the Departments of Anatomy and Medicine, University of California, San Francisco.

ANTIHISTAMINIC VERSUS ANTICHOLINERGIC EFFECTS OF ATROPINE ON CANINE TRACHEALIS MUSCLE

The purpose of this study was to reexamine the antihistaminic and anticholinergic effects of atropine in experiments designed to eliminate possible problems. To determine antihistaminic versus anticholinergic effects of atropine in airway smooth muscle, the investigators used an *in vitro* preparation of canine trachealis muscle strips and determined atropine's effect on contractile responses induced by histamine or by electrical field stimulation of cholinergic nerves. In the first series of experiments, 53 strips had initial responses to field stimulation determined and were then randomly assigned to a control group or to a group treated with atropine before field stimulation was repeated and histamine was given. Atropine in concentrations of 10^{-8}, 10^{-7}, and 10^{-6} M decreased the response to field stimulation to 61.4, 10.5 and 0% of the initial response, respectively, but had no effect on the responses to histamine. In the second series of experiments, 24 strips were treated with indomethacin to prevent histamine tachyphylaxis; these strips had initial responses to both field stimulation and histamine determined and were then assigned to a control group or to a group treated with atropine before field stimulation and histamine were repeated. In these experiments, a concentration of atropine (10^{-6} M), which again completely blocked the response to field stimulation, still had no effect on histamine-induced contraction. The researchers conclude that atropine in a concentration that completely blocks the response to cholinergic nerve stimulation has no antihistaminic effect.

Skoogh, B.-E., *Nadel, J. A.*, Fabbri, L. M., Sheppard, D., and Holtzman, M. J.

American Review of Respiratory Disease 128:603-608, 1983.

Other support: National Heart, Lung and Blood Institute and the Fisons Corporation.

From the Cardiovascular Research Institute and the Departments of Medicine and Physiology, University of California, San Francisco.

TIME COURSE OF AIRW OZONE IN DOGS

In the present study, hyperresponsiveness in an siveness before ozone expo To assess responsiveness, response curves of increas delivered to the airways vs with the dogs awake and at the nose and mouth at a le ppm. For both acetylcholi airway responsiveness incr degree 1 day later and retur authors' previous studies ir ness occurs shortly after ex effect is linked to an acute

Holtzman, M. J., Fabbri, Aizawa, H., and *Nadel, J.*

Journal of Applied Physiol 1983.

Other support: National H

From the Cardiovascular Physiology, University Ca

NEURAL CONTROL OF

Major advances have airway secretion, due in lar cell biology, physiology, airway submucosal glands and are regulated by vagal nonadrenergic noncholine increase gland secretion b cells. Stimulated secretion tic properties. Beta-adren little fluid, high concentra and higher viscosity and lo adrenergic stimulation cau tions, high lysozyme conc pletes granules from serou mucin secretion, probably

Nadel, J. A.

European Journal of Resp

Other support: U. S. Pub Fisons Corporation.

From the Cardiovascular R ogy, University of Califor

TIME COURSE OF AIRWAY HYPERRESPONSIVENESS INDUCED BY OZONE IN DOGS

In the present study, the authors examined the time course of ozone-induced hyperresponsiveness in anesthetized dogs. To do this, they assessed airway responsiveness before ozone exposure and then at 1 h, 1 day, and 1 wk after ozone exposure. To assess responsiveness, the researchers anesthetized the dogs and obtained dose-response curves of increasing concentrations of acetylcholine or histamine aerosols delivered to the airways vs. pulmonary resistance. Ozone exposures were carried out with the dogs awake and at rest in an exposure chamber for 2 h breathing either through the nose and mouth at a level of 2.2 ppm or through a tracheostomy at a level of 1.0 ppm. For both acetylcholine and histamine and for both routes of ozone delivery, airway responsiveness increased most markedly at 1 h after ozone, increased to a lesser degree 1 day later and returned to control levels by 1 wk. The results are similar to the authors' previous studies in humans that showed that ozone-induced hyperresponsiveness occurs shortly after exposure and is rapidly reversible and suggest that the ozone effect is linked to an acute inflammatory response in the airways.

Holtzman, M. J., Fabbri, L. M., Skoogh, B.-E., O'Bryne, P. M., Walters, E. H., Aizawa, H., and *Nadel, J. A.*

Journal of Applied Physiology: Respirat. Environ. Exercise Physiol. 55(4):1232-1236, 1983.

Other support: National Heart, Lung and Blood Institute and the Fisons Corporation.

From the Cardiovascular Research Institute and the Departments of Medicine and Physiology, University California, San Francisco.

NEURAL CONTROL OF AIRWAY SUBMUCOSAL GLAND SECRETION

Major advances have occurred recently in the understanding of the processes of airway secretion, due in large part to the application of modern techniques of anatomy, cell biology, physiology, biochemistry, and pharmacology. This report notes that airway submucosal glands occupy a substantial volume of the large conducting airways and are regulated by vagal muscarinic nerves, alpha- and beta-adrenergic nerves, and nonadrenergic noncholinergic nerves. Vagal nerves modulate various reflexes that increase gland secretion by stimulating release of granules from mucous and serous cells. Stimulated secretions are unaltered from baseline in biochemical and viscoelastic properties. Beta-adrenergic stimulation releases secretions containing relatively little fluid, high concentrations of protein and sulfur, low concentrations of lysozyme and higher viscosity and lower elasticity, and selectively depletes mucous cells. Alpha-adrenergic stimulation causes high fluid flows with low protein and sulfur concentrations, high lysozyme concentrations and low apparent viscosity, and selectively depletes granules from serous cells. Nonadrenergic noncholinergic nerves also stimulate mucin secretion, probably by releasing vasoactive intestinal peptide.

Nadel, J. A.

European Journal of Respiratory Disease 64(suppl 128):322-326, 1983.

Other support: U. S. Public Health Service, Vick Division Research, Inc., and the Fisons Corporation.

From the Cardiovascular Research Institute and Departments of Medicine and Physiology, University of California, San Francisco.

NEUROPEPTIDE TYROSINE (NPY): A NEWLY DISCOVERED PEPTIDE IS PRESENT IN THE MAMMALIAN RESPIRATORY TRACT

Neuropeptide tyrosine (NPY), a newly discovered peptide known to modulate blood vessel diameter and smooth muscle tone, has been found in many mammalian organs. Its distribution is similar to that of sympathetic nerve fibers and NPY immunoreactivity has been found in noradrenergic ganglion cells. In a study of the respiratory tract of four mammalian species — man, cat, guinea pig, and rat — NPY immunoreactivity has been localized to nerve fibers. NPY immunoreactive nerve fibers were found in the adventitia of blood vessels and in the airway smooth muscle. Its distribution was strikingly similar to that of sympathetic nerve fibers as demonstrated by dopamine-β-hydroxylase antibodies. The mean (SD) concentrations of NPY in the guinea pig respiratory tract, as determined by radioimmunoassay of tissue extracts, were: upper trachea 3.3 (0.7), lower trachea 2.0 (0.5), and major bronchus 3.5 (1.1) pmol/g. During developmental studies in man, NPY immunoreactive nerve fibers were first observed at 20 weeks' gestation in the trachea, and fibers gradually extended down into the intrapulmonary airways after birth. NPY immunoreactive nerve fibers have a distribution and developmental pattern similar to that of sympathetic nerve fibers in the respiratory tract. The finding of NPY immunoreactivity in nerve fibers in the mammalian respiratory tract adds to the growing number of peptides having potent biological actions found in this organ, and shows that the lung possesses a rich peptidergic system that may influence pulmonary function.

Sheppard, M. N., *Polak, J. M.*, Allen, J. M. and Bloom, S. R.

Thorax 39:326-330. 1984.

From the Departments of Histochemistry and Medicine, Royal Postgraduate Medical School, Hammersmith Hospital, London, England.

TWO PROTEINASE INHIBITORS ASSOCIATED WITH PERITONEAL MACROPHAGES

In this study, the investigators document and characterize two proteinase inhibitors associated with guinea pig perinoneal macrophages. Results show that inhibition is dose-dependent, lost on heating and detected in the presence of excess albumin. On incubation of macrophage culture medium with ^{125}I-elastase, two complexes of $M_r = 78,000$ and 66,000 are generated which are stable to heating in sodium dodecyl sulfate, indicating covalent association. ^{125}I-Trypsin forms two complexes of similar molecular weight, and cross-inhibition experiments demonstrated that elastase and trypsin interact with the same two macrophage inhibitors. For comparison, elastase inhibitors in guinea pig plasma and cell-free peritoneal fluid were also examined. In summary, the inhibitor which forms the $M_r = 66,000$ complex with ^{125}I-elastase has been tentatively named MPI (macrophage proteinase inhibitor). MPI can be obtained free of α_1PI by culturing macrophages for 1 h. α_1PI is released into the medium during the 1st h of culture and thereafter is no longer detectable on intact macrophages, in subsequent culture media or in cell lysates. These findings suggest that plasma α_1PI is present in peritoneal fluid in high concentrations and is absorbed onto or into macrophages. MPI, on the other hand, appears to be a macrophage product, since it is secreted by macrophages after 1 or 17 h in culture and is present in lysates of macrophages precultured for 1 or 17 h.

Remold-O'Donnell, E

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Remold-O'Donnell, E. and Lewandroski, K.

The Journal of Biological Chemistry 258(5):3251-3257, 1983.

Other support: National Cancer Institute.

From the Center for Blood Research and the Department of Biological Chemistry, Harvard Medical School, Boston.

NEUTROPHILS AND THE ADULT RESPIRATORY DISTRESS SYNDROME

This paper reviews the potential role of neutrophils and their products in the pathogenesis of the acute lung injury characteristic of adult respiratory distress syndrome (ARDS). Neutrophils can elaborate O_2-derived products, proteolytic enzymes and arachidonate products. These substances have dramatic effects on experimental lung preparations. The observation that neutrophils accumulate in the lungs of ARDS patients seems clear, but this finding is obviously not specific for or diagnostic of ARDS. Therefore, despite the presence of potentially toxic neutrophils in the lungs of these patients, the specific role, if any, of neutrophils in clinical ARDS remains to be demonstrated. While basic observations must continue to be made in experimental models, an equal effort is needed to determine if experimental mechanisms of lung injury have any relationship to the pathophysiology of critically-ill ARDS patients. Only when this link is convincingly made can one begin to translate experimental findings into clinical methods for diagnosis and therapy. It is conceivable that some manipulation of neutrophil behavior could favorably affect the pathophysiology of ARDS. But because inflammation is so intimately involved in the normal processes of host defense and tissue injury and repair, therapeutic interventions that affect the inflammatory cascade could be double-edged swords and should only be studied in the most careful fashion. Given the frequency and lethality of ARDS, the cost of conducting such studies seems justified.

Tate, R. M. and *Repine, J. E.*

American Review of Respiratory Disease 128:552-559, 1983.

Other support: National Institutes of Health, American Heart Association of Wyoming, and the Kroc, Hill, Swan, and Kleberg Foundations.

From the Division of Pulmonary Sciences and the Department of Internal Medicine, University of Colorado Health Sciences Center, Denver.

MACROPHAGE EFFECTOR FUNCTION IN PULMONARY OXYGEN TOXICITY: HYPEROXIA DAMAGES AND STIMULATES ALVEOLAR MACROPHAGES TO MAKE AND RELEASE CHEMOTAXINS FOR POLYMORPHONUCLEAR LEUKOCYTES

Macrophages synthesize many secretory products *in vitro* but the stimuli for their production and their pathophysiologic significance *in vivo* are largely unknown. In the present investigation, the authors found that hyperoxia damaged rabbit alveolar macrophages (AM) *in vitro* as manifested by decreased cell numbers, increased lactate dehydrogenase release, and the development of ultrastructural abnormalities that resembled those seen in AM *in situ* or lavaged from lung of rabbits exposed to hyperoxia

in vivo. Hyperoxia also stimulated cultured rabbit AM to release chemotaxins for polymorphonuclear leukocytes (PMN) that were similar in molecular weight to chemotaxins obtained from lung lavages of rabbit exposed to hyperoxia *in vivo*. These results suggest that alveolar macrophage secretory products may play a physiologically relevant role in recruitment of PMN to the lungs in pulmonary oxygen toxicity.

Harada, R. N., Vatter, A. E. and *Repine, J. E.*

Journal of Leukocyte Biology 35:373-383, 1984.

Other support: American Heart Association, American Lung Association, National Institutes of Health, and the Kroc, Hill, Swan and Kleberg Foundations.

From the Webb-Waring Lung Institute and the Pulmonary Division of the University of Colorado Health Sciences Center, Denver.

INTACT HUMAN ERYTHROCYTES PREVENT HYDROGEN PEROXIDE-MEDIATED DAMAGE TO ISOLATED PERFUSED RAT LUNGS AND CULTURED BOVINE PULMONARY ARTERY ENDOTHELIAL CELLS

Acute edematous lung injury, such as that seen in the adult respiratory distress syndrome (ARDS), is an important clinical problem whose pathophysiology is poorly defined. However, recent evidence suggests that toxic oxygen metabolites may contribute to endothelial cell injury and acute edematous lung injury. In this study, addition of untreated or glutaraldehyde-fixed human erythrocytes decreased hydrogen peroxide (H_2O_2)-mediated acute edematous injury in isolated rat lungs, H_2O_2-induced damage to cultured bovine pulmonary artery endothelial cells, and H_2O_2-dependent oxidation of reduced cytochrome C *in vitro*. RBC scavenging of H_2O_2 appeared to be dependent on intracellular glutathione and/or catalase activities. The results suggest that intact erythrocytes can scavenge H_2O_2 and, as a result, protect the lung and possibly other tissues from damage.

Toth, K. M., Clifford, D. P., Berger, E. M., White, C. W., and *Repine, J. E.*

Journal of Clinical Investigation 74:292-295, 1984.

Other support: National Institutes of Health, American Heart Association, American Lung Association, and the Swan, Hill, Kleberg, and R. J. Reynolds Foundations.

From the Departments of Medicine and Pediatrics, and the Webb-Waring Lung Institute, University of Colorado Medical Center, Denver.

OXYGEN METABOLITES STIMULATE THROMBOXANE PRODUCTION AND VASOCONSTRICTION IN ISOLATED SALINE-PERFUSED RABBIT LUNGS

Generation of reactive oxygen metabolites, thromboxane increases and vasoconstriction have been implicated in the pathogenesis of acute edematous lung injury, such as that seen in patients with the Adult Respiratory Distress Syndrome (ARDS), but their interactions are unknown. The investigators hypothesized that reactive O_2 products would stimulate arachidonic acid metabolism in lungs and that vasoactive products of arachidonate, such as the potent vasoconstrictor thromboxane A_2, might then

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mediate O_2-metabolite-induced pulmonary vasoconstriction. They found that O_2 metabolites generated by injection of purine plus xanthine oxidase caused increases in mean pulmonary artery perfusion pressure (27 ± 4mmHg) in isolated perfused lungs. In addition, purine plus xanthine oxidase also caused 30-fold increases in perfusate levels of thromboxane B_2 (the stable metabolite of thromboxane A_2) compared with only two-fold increases in 6-keto-$PGF_{1\alpha}$ (the stable metabolite of prostacyclin). Moreover, prior addition of catalase inhibited both vasoconstriction and the thromboxane B_2 production seen in isolated lungs following injection of purine plus xanthine oxidase. Similarly, pretreatment with cyclooxygenase inhibitors, either aspirin or indomethacin, also completely blocked thromboxane generation and markedly attenuated pressor responses usually seen after purine plus xanthine oxidase. Furthermore, imidazole, a thromboxane synthetase inhibitor, also decreased O_2-metabolite-induced thromboxane generation and vasoconstriction. These results suggested that thromboxane generation might participate in O_2-metabolite-induced vasoconstriction. However, since a significant correlation between thromboxane levels and the degree of vasoconstriction could not be demonstrated, and since addition of superoxide dismutase reduced thromboxane but did not affect the intensity of vasoconstriction, it is possible that thromboxane is not the only vasoactive mediator in this model. The researchers conclude that exposing lungs to O_2 metabolites results in thromboxane generation and that thromboxane is a major mediator of oxidant-induced vasoconstriction.

Tate, R. M., Morris, H. G., Schroeder, W. R. and *Repine, J. E.*

Journal of Clinical Investigation 74:608-613, 1984.

Other support: American Heart Association of Wyoming, National Institutes of Health, and the Swan, Hill, Kleberg, Roche, Thrombrands and Proctor and Gamble Foundations.

From Webb-Waring Lung Institute, and Departments of Medicine and Pediatrics, University of Colorado Health Science Center, Denver.

BRONCHIAL LAVAGE PROTEINS AS CORRELATES OF HISTOPATHOLOGIC AIRWAY CHANGES IN HEALTHY SMOKERS AND PATIENTS WITH PULMONARY CARCINOMA

Cigarette smoking is known to be an important etiologic factor in several lung diseases; however, the number of smokers who develop these diseases represents a small segment of the smoking population. It is possible that evidence of inhalation-induced injury to bronchial epithelial cells of smokers will be reflected in the proteinaceous products of these cells, thereby identifying a high-risk subgroup. The investigations have tested this hypothesis by analysis of 2 proteins, free secretory component (FSC) and the keratins, in lavage fluids obtained from 4 groups of subjects: 30 normal nonsmokers, 15 asymptomatic smokers, 22 symptomatic smokers and 40 carcinoma patients. Among symptomatic smokers, FSC relative to total protein (FSC/TP) was depressed compared with that in nonsmokers and asymptomatic smokers. The keratins were detected only in symptomatic smokers and correlated with pack/years of smoking history ($p = 0.017$). Carcinoma patients had depressed FSC/TP and detectable keratin (33 of 38 patients studied). Lung sections from carcinoma patients studied immunohistochemically revealed an apparent inverse relationship between tissue FSC and keratins. This inverse relationship was borne out by analysis of these proteins in the

lavage fluid of cancer patients ($r = -0.4$, $p = 0.04$). Thus, in cancer patients, immunohistochemical evidence of airway injury correlates with bronchial lavage levels of mucosal epithelial cell proteins. It is possible that smokers with altered levels of these proteins may be the ones at increased risk of smoking-associated lung disease.

Merrill, W. W., Barwick, K. W., Madri, J., Strober, W., Matthay, R. A., Olchowski, J., Naegel, G., and *Reynolds, H. Y.*

American Review of Respiratory Disease 130(5):905-909, 1984.

Other support: National Heart, Lung and Blood Institute and the American Cancer Society.

From the Pulmonary Section, Department of Medicine, Veterans Administration Medical Center, West Haven, the Pulmonary Section, Department of Medicine and Department of Pathology, Yale University School of Medicine, New Haven, CT; and the Clinical Immunology Section, National Cancer Institute, Bethesda, MD.

ELASTIN PRODUCTION BY CULTURED CALF PULMONARY ARTERY ENDOTHELIAL CELLS

The apposition of endothelium to elastic fibers in large arteries and in the gas exchange regions of the lung suggests that endothelial cells have the capacity to synthesize elastin, the integral component of the elastic fiber. In this report, the authors show that endothelial cells isolated from calf pulmonary artery synthesize tropoelastin under appropriate culture conditions. This was demonstrated by radioimmunoassay for soluble elastin, by immunoprecipitation of a tropoelastin-like product from cell culture medium, and by immunofluorescent microscopy utilizing antielastin IgG. Results indicate that endothelial cell tropoelastin shows many similarities with tropoelastin synthesized by other bovine cell types, including comigration on SDS-PAGE with tropoelastin from fibroblasts, chondroblasts, and smooth muscle cells as a single-chain protein with an apparent molecular weight of approximately 70,000. In addition, the cross-reactivity of tropoelastins from endothelial cells, smooth muscle cells and ear chondroblasts with antiserum to ligament elastin indicates close homology between primary elastin gene products in the various elastin-secreting cell types. These results provide compelling evidence that cultured bovine aorta endothelial cells produce elastin when cultured in medium conditioned by smooth muscle cells, and suggest a possible role for smooth muscle cells in the regulation of elastin production by endothelial cells.

Mecham, R. P., Madaras, J., McDonald, J. A., and *Ryan, U.*

Journal of Cellular Physiology 116:282-288, 1983.

Other support: American Heart Association, Missouri Affiliate, U. S. Public Health Service, and the American Heart Association.

From the Pulmonary Division, Washington University School of Medicine at the Jewish Hospital of St. Louis, and the Department of Medicine, University of Miami, Miami, FL.

ENDOTHELIAL CELL

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ENDOTHELIAL CELLS AND INFLAMMATION

This review of endothelial cells and inflammation focuses primarily on research findings since 1979, by which time effects of inflammation on the pulmonary vasculature were recognized as contributing to the formation of a protein- and cell-rich pulmonary edema fluid. On the basis of more recent studies, investigators are now in a position to consider that what happens to the endothelium as a tissue (or to endothelial cells themselves) depends in part on active reaction to injury. The response of endothelium to injury very likely accelerates vascular occlusion in the short term but may provide a basis for repair and resolution of vascular damage in the long term. In a section on mediators of the inflammatory process, attention is given here to: (1) complement components (anaphylatoxins; (2) endothelium and inactivation of the anaphylatoxins; (3) carboxypeptidase N of endothelial cells; (4) toxic oxygen products; (5) other mediators; and (6) molecular and subcellular responses of endothelium to injury. Building on this material, a hypothesis is presented which deals with the sequence of events set in motion when a sensitized animal receives intravenous antigen. While remarkable progress has been made in understanding of cellular, subcellular and molecular mechanisms of endothelial injury and responses to injury, it appears certain, overall, that acute inflammation will not be understood in terms of a single mediator, all mediators taken together, or platelets, neutrophils, macrophages, or endothelial cells taken alone. Rather, improved understanding will likely come from clearer definitions of how all these components and cellular elements interact with the inciting stimulus and then with each other.

Ryan, U. S. and Ryan, J. W.

Clinics in Laboratory Medicine 3(4):577-599, 1983.

Other support: U. S. Public Health Service.

From the Department of Medicine, University of Miami, Miami, FL.

CULTURE OF PULMONARY ENDOTHELIAL CELLS ON MICROCARRIER BEADS

This chapter reviews the techniques available for the isolation and culture of pulmonary endothelial cells and the need and means for monitoring the quality of the cells through successive passages. Culture methods can be divided into those using enzymes and those which avoid exposure of cells to enzymes at both the isolation step and during subculture. The former are by and large routine, while the latter are still at the development stage but present new vistas to be explored. The enzymatic methods include: (1) Isolation of endothelial cells from pulmonary artery by collagenase digestion; (2) Isolation of pulmonary microvascular endothelium by retrograde perfusion with collagenase; and (3) Culture and subculture of pulmonary endothelial cells using trypsin EDTA for passaging. On the other hand, the methods avoiding exposure to proteolytic enzymes include: (1) Mechanical harvest of pulmonary artery endothelium; (2) Isolation of endothelium from the small vessels of the lungs by perfusion with cold saline and microcarrier beads; and (3) Microcarrier cultures: Long term, large-scale culture of pulmonary endothelium avoiding exposure to enzymes during passaging. Previous methods for culture of pulmonary endothelial cells have yielded an abundance of useful data, particularly in helping to confirm the role of the endothelium in the pulmonary processing of vasoactive substances. Now, in addition to their obvious advantages, (large number of cells, economy of media and of personnel), microcarrier

bead cultures may allow researchers to study properties which do not survive long periods of culture and subculture involving exposure to proteolytic enzymes, and to study subtle physiologic modulations of integral membrane components and substructures such as caveolae.

Ryan, U. S.

In: Jaffe, E. A. (ed.): *Biology of the Endothelial Cell*, The Netherlands: Martinus Nijhoff, 1984, chap. 4, pp. 34-50.

Other support: National Institutes of Health.

From the Department of Medicine, University of Miami School of Medicine, Miami, FL.

TRYPSIN-INDUCED AGGREGATION OF BOVINE PULMONARY ARTERY ENDOTHELIAL CELLS CULTURED ON MICROCARRIERS

The authors of this paper studied adherence between "luminal" surfaces of pulmonary artery endothelial cells by standard aggregometry techniques widely used for measuring aggregation of platelets and granulocytes. Using suspensions of bovine pulmonary artery endothelial cells cultured on microcarrier beads, in an aggregometer, they found that trypsin caused endothelial aggregation. The aggregation response occurred at trypsin concentrations as low as 0.001%. The degree of trypsin-induced aggregation indicated by the magnitude of the change in light transmission through the endothelial suspensions was related to the trypsin concentration, reaching a maximum level of trypsin concentrations of 0.01%. The investigators conclude the trypsin, even in very low concentrations, causes adherence between "luminal" surfaces of pulmonary endothelial cells probably because the enzyme destroys cell surface proteins which are necessary to prevent intercellular adherence. The method described here may be useful for studying cell-cell interactions of endothelium.

Brigham, K. L., Meyrick, B. and *Ryan, U. S.*

Tissue & Cell 16(2):167-172, 1984.

Other support: National Institutes of Health, Hugh J. Morgan Fund for Cardiology, The John W. Cooke, Jr., and Laura W. Cooke Fund for Lung Research, and the Upjohn Company.

From the Pulmonary Circulation Center, Departments of Medicine and Pathology, Vanderbilt University School of Medicine, Nashville, TN, and the Department of Medicine, University of Miami School of Medicine, Miami, FL.

IN VIVO AUTORADIOGRAPHIC DEMONSTRATION OF β-ADRENERGIC BINDING SITES IN ADULT RAT TYPE II ALVEOLAR EPITHELIAL CELLS

In this study, adult male rats were injected intravenously with the muscarinic binding probe ^{3}H-quinuclidinyl benzilate (QNB) or the β-adrenergic probe ^{3}H-dihydroalprenolol (DHA). Other rats were pre-treated with an intraperitoneal injection of a 500-fold excess of L-isoproterenol prior to the DHA. Light microscopic autoradiography of 0.5 μm sections of lung from the QNB group demonstrated very little labeling even after 6 months' exposure. In contrast, trachealis smooth muscle from

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these animals contained substantial labeling. Autoradiographs of lung from rats injected with DHA demonstrated labeling which was well localized over alveolar septa and concentrated over the cytoplasm of type II cells. Quantitative analysis of labeling in the DHA groups indicated a significant reduction of labeling in animals treated with L-isoproterenol prior to DHA, in both the alveolar parenchyma in general and over type II cells. The results of this study provide morphologic evidence for the uptake and specific binding of β-adrenergic antagonists by the adult lung *in vivo*, while failing to demonstrate similar binding of a muscarinic probe. In addition, the results demonstrate specific β-adrenergic receptors on type II *in vivo* and substantiate the view of a direct effect of β-adrenergic agonists on alveolar type II cells.

Smith, D. M. and Sidhu, N. K.

Life Sciences 34(6):519-527, 1984.

From the Department of Biological Sciences, Wellesley College, Wellesley, MA.

HOW PHAGOCYTIC LEUKOCYTES MOVE

As this paper notes, a regulated, coordinated movement of the cytoplasm is essential for the function of phagocytes. In these cells, as in muscle cells, the power unit for movement consists of the contractile proteins, actin and myosin, which are concentrated in the region of the cell cortex. In the peripheral cytoplasm, actin fibers may be in a fluid state or they may form a gel network by association with a homodimeric, actin-binding protein. The reversible transformation of the cytoplasm from gel to sol is mediated by a regulatory protein called gelsolin which, when activated by micromolar concentrations of Ca^{2+}, causes shortening of actin fibers, leading to disintegration of the gel network. This gel network reforms if the Ca^{2+} concentration falls below the threshold value for the activation of gelsolin. Ca^{2+}, acting via gelsolin, is a second component in this system; it controls the order of events that start on the plasma membrane of the phagocyte in response to a stimulus, and that are then maintained by an appropriate reaction of the contractile unit. It is to be expected that the elucidation of the molecular mechanisms that release and regulate the movement of cytoplasm in the cell will permit an understanding of factors that interfere with leukocyte function.

Hartwig, J. H., Yin, H. L., and *Stossel, T. P.*

Journal of Clinical Chemistry and Clinical Biochemistry 21(9):535-544, 1983.

Other support: U. S. Public Health Service and the Edwin S. Webster Foundation.

From the Hematology-Oncology Unit, Harvard Medical School; Department of Medicine, Massachusetts General Hospital, Boston.

ISOLATION AND SOME STRUCTURAL AND FUNCTIONAL PROPERTIES OF MACROPHAGE TROPOMYOSIN

Tropomyosin purified from rabbit lung macrophages is very similar in structure to other nonmuscle cell tropomyosins. Reduced and denatured, the protein has two polypeptides which migrate during electrophoresis in sodium dodecyl sulfate on polyacrylamide gels with slightly different mobilities corresponding to apparent M_r's of about 30,000. Following cross-linking by air oxidation in the presence of $CuCl_2$, electrophoresis under nonreducing conditions reveals a single polypeptide of M_r

60,000. Macrophage tropomyosin has an isoelectric point of 4.6 and an amino acid composition similar to other tropomyosins. It contains one cysteine residue per chain. In the electron microscope, macrophage tropomyosin molecules rotary shadowed with platinum and carbon are slender, straight rods, 33 nm in length. Macrophage tropomyosin paracrystals grown in high magnesium concentrations have an axial periodicity of 34 nm. On the basis of yields from purification and from two-dimensional electrophoretic analyses of macrophage extracts, tropomyosin comprises less than 0.2% of the total macrophage protein, a molar ratio of approximately 1 tropomyosin molecule to 75 actin monomers in the cell. Macrophage tropomyosin binds to actin filaments. Macrophage, skeletal muscle and other nonmuscle cell tropomyosins inhibit the fragmentation of actin filaments by the Ca^{++}-gelsolin complex. The finding implies that tropomyosin may have a role in stabilizing actin filaments in vivo.

Fattoum, A., Hartwig, J. H. and *Stossel, T. P.*

Biochemistry 22(5):1187-1193, 1983.

Other support: U. S. Public Health Service.

From the Hematology-Oncology Unit, Massachusetts General Hospital, Department of Medicine, Harvard Medical School, Boston.

THREE-DIMENSIONAL STRUCTURE OF ACTIN FILAMENTS AND OF AN ACTIN GEL MADE WITH ACTIN-BINDING PROTEIN

This paper describes four properties for purified actin filaments and for actin assembled in the presence of macrophage actin-binding protein. To do this, purified muscle actin and mixtures of actin and actin-binding protein were examined in the transmission electron microscope after fixation, critical point drying and rotary shadowing. The three-dimensional structure of the protein assemblies was analyzed by a computer-assisted graphic analysis applicable to generalized filament networks. This analysis yielded information concerning the frequency of filament intersections, the filament length between these intersections, the angle at which filaments branch at these intersections, and the concentration of filaments within a defined volume. Purified actin at a concentration of 1 mg/ml assembled into a uniform mass of long filaments which overlap at random angles between 0 and 90. Actin in the presence of macrophage actin-binding protein assembled into short, straight filaments, organized in a perpendicular branching network. The distance between branch points was inversely related to the molar ratio of actin-binding protein to actin. This distance was what would be predicted if actin filaments grew at right angles off nucleation sites on the two ends of actin-binding protein dimers and then annealed. The results suggest that actin in combination with actin-binding protein self-assembles to form a three-dimensional network resembling the peripheral cytoskeleton of motile cells.

Niederman, R., Amrein, P. C., and Hartwig, J. (*Stossel, T. P.*)

The Journal of Cell Biology 96:1400-1413, 1983.

Other support: U. S. Public Health Service, the Edwin S. Webster Foundation and the Veterans Administration.

From the Hematology-Oncology Unit, Department of Medicine, Massachusetts General Hospital, Boston, and the Veterans Administration Medical Center, West Roxbury, MA.

ISOLATION OF ACTIN-BINI
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Corwin, H. L. and Hartwig,

Developmental Biology 99:61

Other support: U. S. Public
Foundation.

From the Hematology-Oncol
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Zaner, S. K. and *Stossel, T*

The Journal of Biological

Other support: U. S. Publ

From the Hematology-Onc
of Medicine, Harvard Me

ISOLATION OF ACTIN-BINDING PROTEIN AND VILLIN FROM TOAD OOCYTES

Two actin-modulating proteins have been purified from toad oocytes. A high-molecular weight protein, similar in structure and function to macrophage actin-binding protein, accounts for the isotropic actin-crosslinking activity in oocyte homogenates. A calcium-dependent activity in toad oocyte homogenates which shortens actin filaments is accounted for by a 95,000-dalton protein which resembles villin, an actin-severing and -bundling protein of avian epithelial brush borders. In the presence of high ($\geq \mu M$) calcium, this protein shortens actin filaments in a concentration-dependent fashion and stimulates filament assembly when added to monomeric actin. In the absence of calcium the protein promotes the formation of actin filament bundles. Therefore, in the toad oocyte actin can be crosslinked into a network of actin-binding protein. Calcium regulation of the actin network may be mediated by villin. These results are different from those reported in echinoderm eggs.

Corwin, H. L. and Hartwig, J. H. (*Stossel, T. P.*)

Developmental Biology 99:61-74, 1983.

Other support: U. S. Public Health Service and the Elsie O. and Philip D. Sang Foundation.

From the Hematology-Oncology and Renal Units, Harvard Medical School, Department of Medicine, Massachusetts General Hospital, Boston.

PHYSICAL BASIS OF THE RHEOLOGIC PROPERTIES OF F-ACTIN

In the study reported here, the viscoelastic properties of purified rabbit skeletal muscle actin filaments (F-actin) were measured at physiologic ionic strength and pH over a range of concentrations and filament lengths. Although F-actin demonstrated transitory elastic behavior, viscous flow was observed at longer times consistent with a high degree of filament overlap. The compliance was independent of stress over a 4-fold range, implying that the measurement did not disrupt any interfilament "bonds." The dynamic storage modulus increased monotonically with frequency over the range measured, whereas the dynamic loss modulus had a relative minimum and was always less than the dynamic storage modulus. These observations are typical of topologically constrained behavior. The absolute value of the complex dynamic viscosity of F-actin, varied as the -0.8 power of the frequency and at a frequency of 0.1 radians/s was proportional to the product of the weight average filament length raised to the 0.7 power and the concentration. The experimental data agreed well with the predictions of a theory of the rheologic behavior of stiff rods in semidilute solutions. We conclude that the mechanical behavior of pure F-actin solutions can be explained on the basis of the mutual topologic constraints to diffusion of long stiff rods which do not otherwise interact.

Zaner, S. K. and *Stossel, T. P.*

The Journal of Biological Chemistry 258(18):11004-11009, 1983.

Other support: U. S. Public Health Service.

From the Hematology-Oncology Unit, Massachusetts General Hospital, Department of Medicine, Harvard Medical School, Boston.

NEW MECHANISM-BASED SERINE PROTEASE INHIBITORS: INHIBITION OF HUMAN LEUKOCYTE ELASTASE, PORCINE PANCREATIC ELASTASE, HUMAN LEUKOCYTE CATHEPSIN G, AND CHYMOTRYPSIN BY 3-CHLOROISOCOUMARIN AND 3,3-DICHLOROPHTHALIDE

Mechanism-based irreversible inhibitors, which have been reported for porcine pancreatic (PP) elastase and bovine pancreatic chymotrypsin A, include halo enol lactones and 6-chloropyrones. Human leukocyte (HL) elastase and cathepsin G are related serine proteases which are involved in the connective tissue destruction that occurs in emphysema and various inflammatory diseases. Both enzymes are inhibited reversibly by heterocyclic structures such as benzoxazinones and benzisothiazolinones, and this suggested that heterocycles containing masked reactive functionalities might act as mechanism-based irreversible inhibitors for HL elastase and cathepsin G. Therefore, these authors prepared 3-chloroisocoumarin (3-chloro-1H-2-benzopyran-1-one) (1) and 3,3-dichlorophthalide (2) and found them to be potent inhibitors of several serine proteases. In summary, evidence presented here indicates that 1 and 2 are mechanism-based irreversible inhibitors of serine proteases. These are the first demonstrated examples of enzyme-activated inhibitors of HL elastase and cathepsin G. These enzymes have been noted to be major contributors to elastin destruction observed in emphysema. These inhibitors and similar structures may have considerable pharmacologic potential as inhibitors *in vivo*.

Harper, J. W., Henni, K. and *Powers, J. C. (Travis, J.)*

Journal of the American Chemical Society 105:6518-6520, 1983.

Other support: National Institutes of Health.

From the School of Chemistry, Georgia Institute of Technology, Atlanta.

MAMMALIAN TISSUE TRYPSIN-LIKE ENZYMES: COMPARATIVE REACTIVITIES OF HUMAN LUNG TRYPTASE, AND BOVINE TRYPSIN WITH PEPTIDE 4-NITROANILIDE AND THIOESTER SUBSTRATES

The subsite specificity of human lung and skin tryptase (trypsin-like enzyme) has been studied at pH 7.5 using 17 amino acid and dipeptide thioester substrates and 14 tripeptide 4-nitroanilide substrates. The reactivity and specificity of the human tryptases were compared with bovine trypsin and other trypsin-like enzymes. Neither tryptase was similar to either kallikrein or factor XII, (Hageman factor). The skin enzyme was the most reactive as measured by the specificity constant k_{cat}/K_M. The best substrate was benzyloxycarbonyl(Z)-Lys-Arg-S-$CH_2CH(CH_3)_2$ which had a k_{cat}/K_M value of 59,000,000 $M^{-1}S^{-1}$. Only a single substrate, Z-Glu-Phe-Arg-4-nitroanilide, was slightly more reactive with the lung tryptase. Both enzymes have extended substrate-binding sites and proline residues at P_3 substantially decrease k_{cat}/K_M. Both enzymes preferred the tripeptide 4-nitroanilides with a P_2 Gly residue over Phe, and both favored the substrate Z-Lys-Gly-Arg-4-nitroanilide over similar substrates containing six other representative amino acid residues at P_2. The lung enzyme was inhibited over three times faster by p-amidinophenyl-methanesulfonyl fluoride than the skin enzyme. The preference of the skin tryptase for substrates with two terminal basic residues indicates that this enzyme could process prohormones and proproteins which contain this structural feature at the cleavage site. The substrates reported in this paper should be use
function of tryptases.

Tanaka, T., McRae, B. J.
Powers, J. D. (Travis, J.)

The Journal of Biological

Other support: National In

From the School of Chemis
ment of Dermatology, Uni
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III

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Warnick, G. R., Benders

Clinical Chemistry 29(3):

From the Northwest Lipid
cine, Seattle.

this paper should be useful for the further characterization of the physiologic function of tryptases.

Tanaka, T., McRae, B. J., Cho, K., Cook, R., Fraki, J. E., *Johnson, D. A.*, and *Powers, J. D.* (*Travis, J.*)

The Journal of Biological Chemistry 258(22):13552-13557, 1983.

Other support: National Institutes of Health.

From the School of Chemistry, Georgia Institute of Technology, Atlanta; the Department of Dermatology, University of Kuopio, Kuopio, Finland; and the Department of Biochemistry, College of Medicine, East Tennessee State University, Johnson City.

III. *Heart and Circulation*

INTERLABORATORY PROFICIENCY SURVEY OF HIGH-DENSITY LIPOPROTEIN CHOLESTEROL MEASUREMENT

Accuracy in the quantification of high-density lipoprotein (HDL) cholesterol is important because measured values are generally interpreted, not in relation to a laboratory's own reference interval but in relation to epidemiological data, when a patient's risk of coronary artery disease is being assessed. Likewise, good precision is important because relatively large differences in risk are predicted by small changes in HDL cholesterol. According to the work presented here, proficiency surveys of Seattle-area laboratories suggest only slight improvement in overall performance in HDL measurement between 1978 and 1982, although the reported workload for HDL has increased by 15%. The mean interlaboratory SD was 64 mg/L in 1982, compared with 79 mg/L in 1978-79. Of the individual laboratory results in the current survey, 39% deviated by more than 50 mg/L from target values as compared with 37% in 1978-79. The discrepant values were primarily ascribable to method inaccuracy. For within-run precision, 80% of laboratories in 1982 had SDs of <30 mg/L, *vs.* 70% in 1978. The 1982 survey included a lyophilized serum prepared by spray freezing in bulk lyophilization (Hyland Omega), identical to the pools used in the College of American Pathologists Comprehensive Chemistry Survey, and five pools of frozen plasma. Interlaboratory variation and biases for the Omega pool were similar to those for the frozen pools.

Warnick, G. R., Benderson, J. M. and *Albers, J. J.*

Clinical Chemistry 29(3):516-519, 1983.

From the Northwest Lipid Research Clinic, University of Washington School of Medicine, Seattle.